Consequences of the Calcite Skeletons of Planktonic Echinoderm Larvae for Orientation, Swimming, and Shape

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Abstract. How the echinoderm larval skeleton is used for support of larval arms, passive orientation, and swimming was examined by experimentally removing the skeletons of plutei and by comparing feeding larvae from four echinoderm classes. All four types of echinoderm larvae oriented with their anterior ends upward in still water, but removing the skeletons of both live and dead four-armed echinoplutei demonstrated that their skeletons enhanced passive vertical orientation with their anterior ends upward. In comparisons of dead four-armed echinoplutei with and without skeletons, the skeleton contributed more than half of the excess density and sinking speed. In comparisons of all four types of feeding echinoderm larvae, larvae with a greater volume of skeleton and a smaller volume of tissues and body cavity were densest. The calculated work necessary to prevent the plutei from sinking was much less than 1% of the total aerobic energy expenditure. Thus calcite skeletons are not essential for passive vertical orientation by echinoderm larvae but enhance it, and the increased density and sinking rates impose little energetic cost in locomotion.

The evolution of larval skeletons may have been influenced by the benefits of passive orientation and by the low costs of swimming with a skeleton. Whatever the primary function of skeletons, the position and form of skeletal elements is influenced by the functional requirement for higher mass posteriorly for passive orientation. Features that enhance passive vertical orientation include posterior ossicles and skeletal rods, posterior thickening of skeletal rods, and formation of juvenile parts near the posterior ends of larvae.

Introduction

Some echinoderm larvae (echinoplutei, ophioplutei) have a skeleton with calcite rods that support projecting arms, but others (auriculariae, bipinnariae) do not. It is peculiar that any small gelatinous planktonic animals should have an internal mineral skeleton for body support, but a supporting function for some internal skeletal elements is obvious. Calcite rods support the soft parts of a pluteus against the contraction of the muscles that dilate its mouth during the rejection of large particles from its oral cavity (Strathmann, 1971). Calcite rods provide the attachment and leverage for muscles that spread the arms of some plutei, presumably in defense (Mortensen, 1921, 1938). Other supporting functions are also likely. Gustafson and Wolpert (1961) suggested that the arm rods of plutei support an epidermis under tension. Emlet (1982, 1983) measured the stiffness of calcite skeletal rods of plutei and analyzed the forces that swimming imposes on projecting arms. He concluded that fenestrated skeletal rods are stiffer than needed to support the arms against forces generated in ciliary swimming and suggested that the stiffness may protect the larvae against predators.

Some calcite skeletal elements, however, do not support soft parts. Some, such as the posterior ossicle in the auricularia, are only solitary lumps that have no imaginable supporting function. Also, the posterior ends of the body rods of four-armed echinoplutei are much thicker

than the arm rods but appear to be subject to no greater forces and are resorbed at later stages. Runnström (1918) and Pennington and Emlet (1986) suggested that the body skeleton of the echinopluteus is a counterweight that orients the larva with its arms upward. Echinoderm larvae have no known statocyst or organ for sensing gravity, yet all feeding larvae tend to swim with their anterior ends upward. Mechanisms enhancing passive orientation may help them maintain a favorable position in the water column.

We are concerned here with the hypothesis that passive vertical orientation has been an important functional requirement in the evolution of echinoderm larval body plans and especially of larval skeletons. That requirements for passive vertical orientation might constrain larval body plans or entail ecological consequences has been little appreciated or studied.

If the skeleton contributes substantially to passive vertical orientation, it also contributes to the excess density of the larva. Excess density is the difference between the density of an object and the density of seawater. Excess mass is the excess density of an object times its volume. The larval center of gravity is the center of its excess mass, and its center of buoyancy is the center of its volume. The weight of a body acts at the center of gravity, and the upthrust from weight of fluid displaced acts at the center of buoyancy (Alexander, 1968). If the skeleton orients the larva passively with anterior end upward, then the larva's center of gravity, as controlled by the skeleton and other parts, must be posterior to the center of buoyancy.

If the density of the soft parts (tissues and body cavities) of a larva was equal to that of seawater, then the effect of the skeleton on passive vertical orientation might be deduced from anatomical measurements of skeletal components alone. However, some soft parts are denser than seawater; some may be less dense than seawater; and the volume of soft parts greatly exceeds the volume of skeleton. For example, Pennington and Emlet (1986) found that four- and six-armed echinoplutei of *Dendraster excentricus* were denser than eight-armed plutei or even newly metamorphosed juveniles and suggested that accumulation of lipids in the gut (Burke, 1981) might account for the lower density at advanced stages. In addition, the density of the contents of the primary body cavity may differ from the density of cells, and the primary body cavity can be small or extensive, depending on the stage and type of larva. Thus passive vertical orientation by an appropriate distribution of density along the body axis would constrain the distribution of cells, body cavities, and skeleton. Demonstrating a role of the skeleton in passive orientation therefore requires more than anatomical measurements.

In this study we examined the calcite skeletons of planktotrophic echinoderm larvae to identify those functional consequences of skeletal size and shape that may have influenced the evolution of these larval forms. To examine the role of calcite skeletons in passive vertical orientation, we (1) experimentally removed skeletons from echinoplutei and (2) compared types of planktotrophic echinoderm larvae that differ in skeletal development. These studies also addressed two related topics: the role of the calcite skeletons in the maintenance of body shape, and the contribution of calcite skeletons to excess density and sinking rate.

Materials and Methods

Rearing

The methods of obtaining gametes, fertilizing ova, and maintaining embryos and larvae are those in Strathmann (1987). Most larvae were reared in 1.5 to 3 liter cultures in mechanically stirred jars. Some cultures of 0- to 4-day-old larvae were kept in smaller containers and not stirred. Rearing temperatures were about 10–14°C at the Friday Harbor Laboratories on San Juan Island and 23–27°C at the Kewalo Marine Laboratory on Oahu, within a few degrees centigrade of the local sea temperatures.

At the Kewalo Marine Laboratory, larvae were fed *Rhodomonas lens* (as described in Leahy, 1986), occasionally supplemented with *Dunaliella tertiolecta*. These larvae were the echinoplutei of *Echinometra mathaei* (Blainville), *Tripneustes gratilla* (Linnaeus), and *Colobocentrotus atratus* (Linnaeus).

Most larvae at the Friday Harbor Laboratories were fed natural phytoplankton supplemented with *Dunaliella tertiolecta* and occasionally also *Thalassiosira weissflogii* or *Isochrysis galbana*. These larvae were the auriculariae of *Parastichopus californicus* (Stimpson), the bipinnariae of *Asterina miniata* (Brandt) and *Evasterias troschelii* (Stimpson), the ophioplutei of *Ophiura sarsi* Lütken and *Ophiopholis aculeata* (Linnaeus), and the echinoplutei of *Strongylocentrotus droebachiensis* (O. F. Müller), *S. pallidus* (G. O. Sars), *S. purpuratus* (Stimpson), and *Dendraster excentricus* (Eschscholtz).

Killing

Dead larvae were required for several experiments. At Kewalo Marine Laboratory larvae were killed with 2% formalin buffered with calcium carbonate in seawater. At the Friday Harbor Laboratories larvae were killed with sodium cyanide (about 0.005 to 0.001 M) in seawater.

Decalcification

Different methods were used to decalcify dead or live echinoplutei. The skeletons of dead, formalin-fixed lar-

vae were removed by placing the larvae in a 1:1 mixture of seawater and EDTA-saturated seawater for 2–10 min. Complete decalcification was verified by viewing larvae under a dissection microscope with crossed polarizing filters: any remaining skeleton produced a bright glow.

The skeletons of live echinoplutei were removed by the method of Pennington and Hadfield (1989). Batches of several hundred early four-armed plutei (3–5 days old) were pipetted into synthetic seawater (MBL recipe; Cavanaugh, 1956) buffered at pH 5.5 or 6.0 with a final concentration of 0.03 M MES (2-[N-Morpholino]ethanesulfonic acid). Although the rate of decalcification varied with the species, number, and stage of larvae, the plutei usually decalcified in 2 to 5 h; they were then removed to natural seawater. Because this treatment temporarily suppressed motility, the larvae were allowed to recover overnight before behavioral observations were made. Decalcified echinoplutei were maintained in groups of 1–50 larvae in 20-ml finger-bowls and fed *R. lens*.

Orientation, density, and sinking

Orientations and relative densities were compared for dead larvae sinking in density gradients. At Friday Harbor at least three and at Kewalo at least two larvae of each type were pipetted into the top of cuvettes about 3 cm tall by 1 cm wide and deep or in some cases about 10 cm high by 1.3 cm wide and deep. The cuvettes contained seawater at the top and a denser fluid at the bottom; partial mixing of the two fluids produced a density gradient. Except where noted, the denser fluid was Percoll in a solution of NaCl isosmotic with seawater. Percoll is a colloidal suspension of silica particles coated with polyvinylpyrrolidine and was obtained from Pharmacia. Some observations were repeated with sucrose isosmotic with seawater as the denser solution. Resting orientation at neutral buoyancy was checked by reorienting the larva with a glass needle and then waiting several minutes to see if it would return to its original position. (The needle was swept past the larva so that the return stroke did not rotate the larva back to its original position.)

The densities of echinoplutei with and without skeletons were estimated in three experiments. Dead four-armed plutei of *T. gratilla* (4–5 days old) were suspended in seawater layered over a series of Percoll dilutions of decreasing specific gravity but isosmotic with seawater. If larvae floated above the Percoll for 30 min, their density was recorded as less than that of the Percoll solution; if they sank to the bottom, their density was greater than that of the Percoll solution. The densities of the Percoll dilutions were obtained by weighing known volumes. Isosmotic Percoll solutions were used instead of the su-

crose solutions of Pennington and Emlet (1986), who used sucrose solutions without adjusting osmolarity. When the Percoll method was compared with sucrose solutions of unadjusted osmolarity for both normal and decalcified plutei of *T. gratilla*, higher estimates of larval density were produced with the sucrose.

To compare the orientation and sinking rates of echinoplutei with and without skeletons, we used four-armed plutei of *T. gratilla* (3 and 5 days old). Observations of dead larvae with and without skeletons were made in cuvettes, as described above. In seawater without a density gradient, currents were eliminated by minimizing air currents, covering the cuvettes, using indirect backlighting, and equilibrating air and water temperatures. Motionless suspended particles indicated an absence of currents. These observations may underestimate sinking rates because of wall effects in the small cuvettes. Larger cuvettes in water baths were impractical because plutei without skeletons were difficult to see. We measured the sinking rates of larvae that were away from the walls. The observations were adequate to demonstrate an effect of the skeleton and to provide a check on other measurements of the contribution of the skeleton to the density of the larval body.

Stability of vertical orientation was compared for echinoplutei with and without skeletons. Convection currents in the cuvettes were enhanced by shining a lamp on the cuvette wall so that the current rose on one side and descended on the other. Dead four-armed echinoplutei of *T. gratilla* (3 days old), either with or without skeletons, were pipetted into the top of the descending current. The vertical orientations of the first larvae carried upward past mid-depth in the ascending current were scored as (1) arms-up, (2) arms-down, or (3) horizontal.

Orientation during swimming was compared for live echinoplutei with and without skeletons. Decalcified and normal plutei of *T. gratilla* and *C. atratus* were videotaped. Upward swimming indicated orientation to gravity. Swimming in other directions or tumbling indicated deficient orientation to gravity.

Results

Effects of experimental removal of skeletons

Shape. Decalcified dead echinoplutei retained the pluteus form despite the complete loss of skeleton (Fig. 1).

Decalcified live echinoplutei of *E. mathaei*, *T. gratilla*, and *C. atratus* were maintained for 74, 48, and 19 days, respectively. The larval shape changed during this time. Larvae without skeletons initially retained some features of the pluteus shape (Fig. 2A, B). Larval arms were shorter than arms of control plutei with skeletons (Fig. 2C) but were still present (Fig. 2B). Arms without a sup-
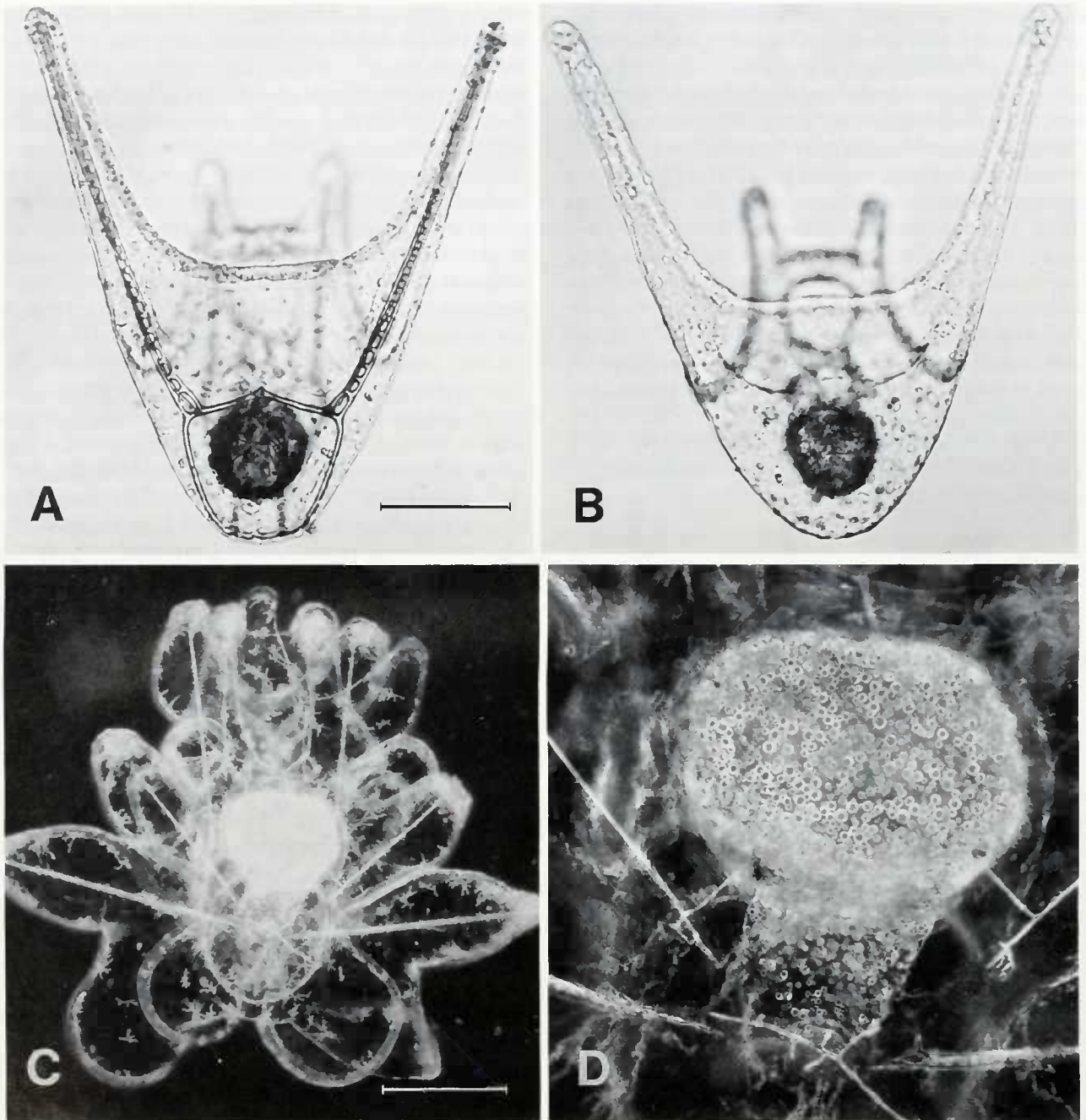
Figure 1. Echinoplutei of *Tripneustes gratilla*, 6 days old: (A) normal control larva with 100 µm scale bar and (B) larva killed with formalin and decalcified with EDTA, same scale. Late eight-armed echinopluteus of *Tripneustes gratilla* with echinus rudiment: (C) whole larvae with 300 µm scale bar and (D) stomach with refractile droplets that are absent in the stomach wall of larvae at earlier stages (3 times the magnification of C).

porting skeleton did not bend noticeably during swimming. The esophagus was initially constricted in *T. gratilla,* but later opened. The mouth, stomach, and intestine appeared normal in all three species, and the larvae without skeletons captured and ingested phytoplankton cells. Some plutei did not resecrete a skeleton, and they

completely resorbed their arms over the course of several days. These larvae became bowl or saucer shaped but retained distinct oral hoods and concave circumoral fields (Fig. 2D). The ciliated band persisted as the rim of the bowl. However, most larvae resecreted abnormal skeletons (Fig. 2E, F). Their arms developed to various
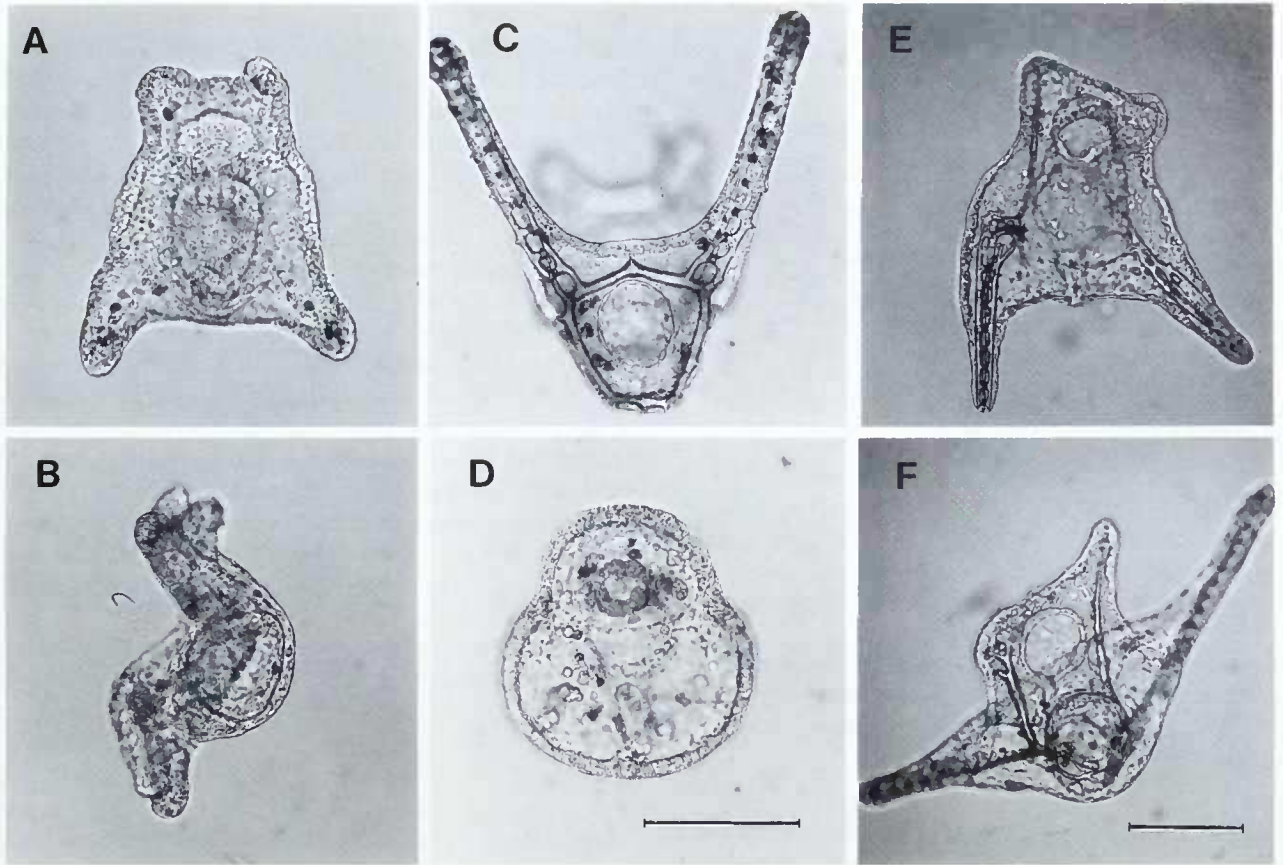
Figure 2. Live four-armed echinoplutei of *Colobocentrotus atratus:* (A) oral and (B) lateral views of recently decalcified plutei, (C) ventral view of normal control larva of same age as larvae in A and B, (D) oral view of larva that had been decalcified several days previously and did not regrow a skeleton, (E) and (F) oral views of plutei that had been decalcified several days previously and did regrow a skeleton.

lengths and in odd directions, and their body skeletons were equally abnormal. Most developed four arms, the correct number, though some had missing or extra arms. In *C. atratus* and *T. gratilla*, the postoral arm rods were usually fenestrated as in normal control larvae. No decalcified larvae developed to six-armed or later stages, but conditions in 20-ml culture dishes were not favorable.

Skeletons in echinoplutei appeared to play a small role in immediate mechanical support and a greater role in long-term maintenance and development of larval form.

Passive orientation. In still seawater, almost all four-armed echinoplutei of *T. gratilla* with and without skeletons oriented passively with their arms upward. Nearly 100% of dead larvae with skeletons sank with arms upward, and about 80% of those without skeletons sank with arms upward. However, removal of the skeleton did change the stability in the arms upward orientation. In a cuvette with 100% seawater and side illumination, there were convection currents and velocity gradients; dead echinoplutei with skeletons were oriented arms upward, but dead echinoplutei without skeletons tumbled. Of lar-

vae at mid-depth in the ascending current, 100% of those with skeletons were arms-upward (n = 30) and only 28% of those without skeletons were arms-upward with the remainder arms-downward or horizontal (n = 40). We expected that with random orientation about 25% of larvae should have been arms upward. The plutei without skeletons did not differ significantly from this expectation (Chi-square, $P > 0.9$), while the plutei with skeletons were clearly non-random in their orientation ($P < 0.005$). Removal of the skeleton greatly decreased passive stability.

Orientation of living echinoplutei. The vertical orientation of live four-armed echinoplutei of *C. atratus, E. mathaei,* and *T. gratilla* was compared with and without skeletons. Most plutei with skeletons swam with their arms upward. Forward swimming produced upwards movement. The arms-upward orientation was less pronounced after decalcification. Plutei of *C. atratus* were the most vigorous after decalcification. Almost all of these plutei tumbled in an anterior-posterior direction with little or no net movement. A few decalcified plutei

Table I

Ranking of density of some Hawaiian echinoplutei from least dense to most dense with larvae of nearly equal density lumped at the same rank

Rank	Species	Stage	Reorientation
1	*Tripneustes gratilla*	four-armed decalcified	*
1	*Tripneustes gratilla*	eight-armed with skeleton	*
2	*Echinometra mathaei*	eight-armed with skeleton	*
3	*Tripneustes gratilla*	four-armed with skeleton	*
3	*Colobocentrotus atratus*	eight-armed with skeleton	*

* = Those that reoriented as they sank toward neutral buoyancy.

did swim forward without tumbling, though they swam downwards as well as upwards with no clearly discernible orientation to gravity. When disturbed, larvae with and without skeletons stopped or swam backwards. Results with *E. mathaei* and *T. gratilla* were similar, though decalcified larvae of these species swam less vigorously.

Density. When the skeletons of four-armed (4 and 5 day old) echinoplutei of *T. gratilla* were removed, the density of the larval body decreased. The density of larvae with skeletons was estimated to be about 1.06 g/ml, because all dead larvae with skeletons sank through a Percoll solution of density 1.042 g/ml, and all floated above a solution of density greater than 1.078 g/ml. More than half (*ca.* 70%) sank in a solution of density 1.054 g/ml. The density of larvae without skeletons was estimated to be about 1.04 g/ml because all dead larvae without skeletons sank through a solution of density 1.037 g/ml and all floated in a solution of density 1.042 g/ml.

In a density gradient, the terminal depth of four-armed plutei of *T. gratilla* without skeletons was less than the depth of control plutei with skeletons (Table 1). This result again indicates that the skeleton increases the density of the larval body.

Sinking speed. Removal of skeletons of four-armed echinoplutei of *T. gratilla* decreased their sinking rates in seawater. When 3 days old, echinoplutei with skeletons sank at 0.29 mm/s (S.D. = 0.076 mm/s, n = 10), and plutei without skeletons sank at 0.052 mm/s (S.D. = 0.022 mm/s, n = 10). When 5 days old, echinoplutei with skeletons sank at 0.44 mm/s (S.D. = 0.21 mm/s, n = 10), and plutei without skeletons sank at 0.052 mm/s (S.D. = 0.018 mm/s, n = 10). In both comparisons the sinking speeds were significantly less for plutei without skeletons (*t*-test, $P < 0.001$).

Comparisons of larval forms and stages

Passive orientation. Echinoderm larvae of four classes passively oriented with their anterior ends upward while sinking in seawater at the top of Percoll gradients. The

dead larvae that sank with their anterior ends upward were auriculariae of *P. californicus*, bipinnariae of *A. miniata* and *E. troschelii*, ophioplutei of *O. aculeata* (four-armed) and *O. sarsi* (six-armed), and echinoplutei of *D. excentricus* (four- and eight-armed), *S. franciscanus* (four- and eight-armed), *S. pallidus* (four-, six-, and eight-armed), *S. purpuratus* (four-armed), *C. atratus* (eight-armed), and *T. gratilla* (four-armed). All were tested in gradients made with seawater as the less dense solution and with a mixture of Percoll and isosmotic NaCl as the denser solution. Many were also tested in gradients made with seawater and an isosmotic sucrose solution, with no difference in results. At the seawater end of the density gradient, sinking dead larvae reoriented with their anterior ends upward almost immediately after being turned anterior end downward. The two exceptions were eight-armed echinoplutei of *T. gratilla* and *E. mathaei*, which did not clearly orient with their arms upward while sinking into the top of a Percoll gradient.

Dead larvae of all four types also oriented anterior end upward when sinking in 100% seawater with no density gradient, except that the larvae were sometimes turned by the convection currents that developed under uncontrolled conditions in cuvettes lacking a density gradient.

Sinking was not required for passive orientation. Orientation was stable for larvae suspended at neutral buoyancy. However, some larval forms reversed their orientation as they sank into denser fluid because the distribution of excess mass was different in denser media. These results are addressed below, following the observations on density.

Density. Calcite skeletons affected larval density. In a density gradient, dead larvae with more skeleton in proportion to body volume came to rest in denser fluid (Tables 1, II); larvae with little or no skeleton (auricularia and bipinnaria, Fig. 3A, B) or with the skeleton removed (pluteus of *T. gratilla*, Fig. 1B) were least dense. For comparisons among a range of larval forms, the larvae in Table II were ranked according to the proportion of body volume composed of calcite skeleton: 1 for the bipinnariae, with no skeleton; 2 for the auriculariae, with only a small ossicle; 3 for the plutei of *Strongylocentrotus* from four- to early eight-armed stages, with thin skeletal rods, an incomplete basket skeleton, and large spaces between skeleton and body wall; and 4 for the remaining plutei, with a relatively large skeleton because of thick rods, fenestrated rods, a more complete basket skeleton, or juvenile skeletal elements of the later rudiment. These ranks for skeleton as a proportion of body volume were correlated with the whole body densities in Table II (Spearman rank correlation 0.86, $P < 0.01$). These rank data comprise a broad range of forms of planktotrophic echinoderm larvae.
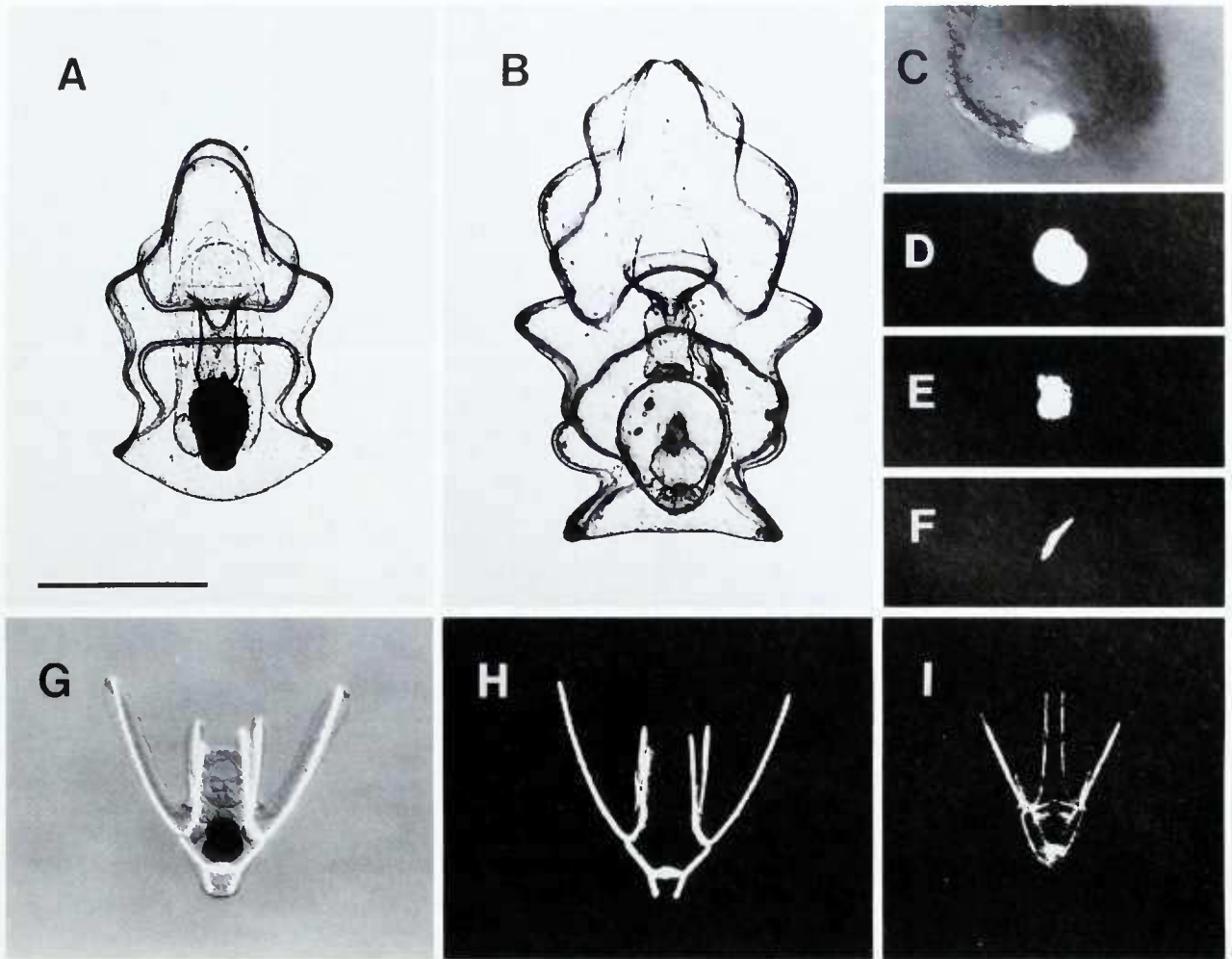
Figure 3. (A) Bipinnaria of *Asterina miniata*, (B) auricularia of *Parastichopus californicus*, (C, D, E) view of broad side and (F) view of narrow side of ossicles at the posterior, left corner of the body of *P. californicus*, (G) and (H) ophiopluteus of *Ophiura sarsi*, (I) echinopluteus of *Dendraster excentricus*. Calcite skeletons shown with polarizing filters partially crossed in (C) and (G) and fully crossed in (D, E, F, H, I). Scale bar is 400 μm in (A, B, G, H, I) and 160 μm in (C, D, E, F).

Among echinoplutei, the stage of development affected density. In a sample of 32 larvae of *S. franciscanus*, the eight-armed larvae with two or fewer pedicellariae and no juvenile spines or plates (Fig. 4E) were less dense than both the earlier stage four- to six-armed larvae (Fig. 4C, D) and the more advanced stage eight-armed larvae with three pedicellariae (Fig. 4F, G). (Juvenile plates and spines were forming in some of the larvae with three pedicellariae.) The differences in density among stages were statistically significant ($P < 0.001$, Kruskal-Wallis test). Eight-armed plutei were similarly less dense than four-armed plutei within the species *T. gratilla* (Table I, Fig. 1A, C) and *D. excentricus* (Table II). Very advanced eight-armed plutei were more dense than four-armed plutei in *S. pallidus* (Table II, Fig. 4H). The density changes during development were what one would

have predicted from skeletal development. In early eight-armed stages of *Strongylocentrotus* species, the volume of skeleton relative to the volume of soft parts had decreased because the heavy ends of the body rods had disappeared and because the soft parts had grown more than the skeletal rods (Fig. 4). Also in *T. gratilla*, the plutei at the eight-armed stage (Fig. 1C) were much more fleshy than the plutei at the four-armed stage (Fig. 1A), and the density of eight-armed *T. gratilla* was almost as low as the density of the decalcified four-armed plutei (Table I). In these comparisons among stages, greater development of skeleton relative to soft parts was associated with greater density. However, Pennington and Emlet (1986) did not find an increase in density during rudiment development of larval *D. excentricus*. They suggested that lipids in cells of the gut (Burke, 1981)

Table II

Ranking of density of larvae from least dense to most dense with approximately equally dense larvae lumped together

Rank	Form	Species	Stage	Reorientation
1	auricularia	*Parastichopus californicus*		
1	bipinnaria	*Asterina miniata*		
2	echinopluteus	*Strongylocentrotus franciscanus*	eight-armed, small echinus rudiment	*
3	echinopluteus	*Strongylocentrotus franciscanus*	four-armed	*
3	echinopluteus	*Strongylocentrotus pallidus*	four-armed	*
4	echinopluteus	*Strongylocentrotus pallidus*	eight-armed, advanced echinus rudiment and postlarval plates and spines	*
5	echinopluteus	*Dendraster excentricus*	eight-armed	
6	echinopluteus	*Dendraster excentricus*	four-armed	
7	ophiopluteus	*Ophiopholis aculeata*	four-armed	

* = Those that reoriented as they sank toward neutral buoyancy.

might compensate for skeletal development at this stage. Refractile droplets appeared in the stomach cells of nearly competent plutei of *T. gratilla* (Fig. 1D), but we were not able to compare the specific gravities of all stages of *T. gratilla.*

Differences in whole body density among plutei of different species (Table II) were related to larval shape and skeletal development. The early four-armed pluteus of *S. purpuratus* (Fig. 4A, B) was denser than the early four-armed pluteus of *S. pallidus. S. purpuratus* develops from a smaller egg than does *S. pallidus,* and its pluteus begins with a larger skeleton relative to its body volume. Larvae of *S. purpuratus* converge on the form of other *Strongylocentrotus* larvae as they grow (Sinervo and McEdward, 1988), and presumably they converge in density at later stages also. Echinoplutei of *D. excentricus* have thick fenestrated arm rods and an extensive body skeleton (Fig. 3I) and, as expected, were denser than plutei of *Strongylocentrotus.* Four-armed ophioplutei of *O. aculeata* were the densest larvae examined (Table II), and ophioplutei in general have thick skeletal rods and a relatively small body cavity (Fig. 2G, H). (Plutei of *S. purpuratus* were not tested against plutei of *D. excentricus* or *O. aculeata* and therefore could not be included in Table II.) These comparisons indicate that the ratio of skeleton to whole body volume is a major determinant of density of the whole larval body.

Some differences in skeletons were too small to override other determinants of density of the larvae. The auriculariae of *P. californicus,* with their small posterior ossicles (Fig. 3B, C), and the bipinnariae of *A. miniata,* with no skeleton (Fig. 3A), sank to about the same depths in the density gradient. The ossicles of the auriculariae made no discernible difference in overall densities of the larval bodies. The auriculariae and bipinnariae had different lengths and body volumes, and this could have affected overall body density more than a small ossicle.

Similarly, in comparisons among auriculariae of *P. californicus,* density of the larval body was not discernibly affected by the presence or absence of a posterior ossicle, but it did vary with larval size. Size, shape, and location of posterior ossicles varies; so in a sample of 30 auriculariae from 2 pairs of parents, 7 auriculariae lacked any posterior ossicle, 20 had a posterior left ossicle, and 3 had an additional posterior right ossicle. In this sample there was no relation between density of the larval body (depth of neutral buoyancy in a Percoll gradient) and the occurrence of posterior ossicles. Most posterior ossicles were broad in two dimensions (commonly 30 to 50 μm) but thin in the third (about 10 μm) (Fig. 3D, E, F). Volumes of ossicles ranged from about 5,000 to 13,000 μm^3 (calculated as spheroids; sample of 4 larvae).

In the sample of 30 auriculariae, longer larvae were less dense than short larvae ($P < 0.02$, two-tailed rank-sum test, and Spearman rank correlation 0.59, $P < 0.001$). Longer auriculariae were also less dense in a comparison of 12 auriculariae, all from the same pair of parents and ranging in length from 650 to 870 μm ($P < 0.02$, two-tailed rank-sum test, and Spearman rank correlation 0.95, $P < 0.001$). Larger echinoderm larvae have larger primary body cavities but about the same thickness of body wall (McEdward, 1984), which is a single layer of cells. As an approximation, the volume of the body cavity should increase in proportion to the cube of body length and the volume of body wall in proportion to the square of length. The lower densities of longer auriculariae were therefore consistent with the hypothesis that their cells are denser than the contents of their primary body cavity, but other aspects of larval condition could have been confounded with size.

Effect of density of surrounding fluid on orientation

Passive orientation depends on the density of the surrounding fluid. All dead larvae passively oriented ante-
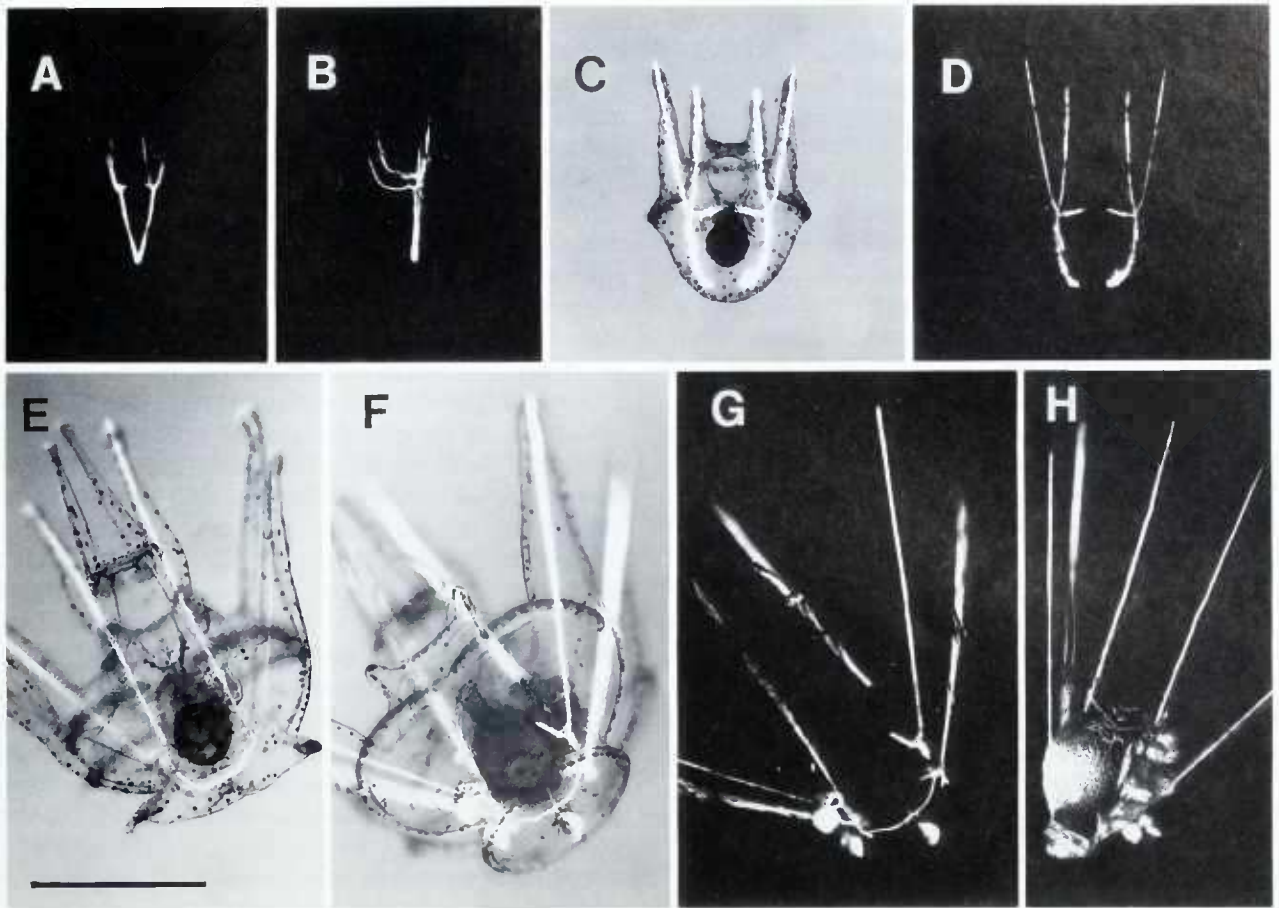
Figure 4. Echinoplutei of *Strongylocentrotus* species: (A) ventral and (B) lateral views of four-armed
S. purpuratus, (C) and (D) four-armed *S. franciscanus*, (E) eight-armed *S. franciscanus* without pedicel-
lariae, (F) and (G) eight-armed *S. franciscanus* with three pedicellariae, and (H) eight-armed *S. pallidus*
with juvenile plates and spines. Calcite skeleton shown with polarizing filters partly crossed in (C, E, F)
fully crossed in (A, B, D, G, H). Because of their optical axis the preoral arm rods are not shown. Scale bar
is 400 μm for all photos.

rior end upward when in seawater or in low-density mix-
tures of seawater and isosmotic NaCl and Percoll or in
seawater and isosmotic sucrose. However, echinoplutei
of *C. atratus, E. mathei, T. gratilla, S. franciscanus,* and
S. pallidus reoriented with their arms downward as they
sank into the denser fluid in the density gradients and
approached the depth at which they were neutrally buoy-
ant (Tables I, II). The four-armed plutei of *S. purpuratus*
and the plutei of *D. excentricus* and *O. aculeata* did not
reorient at neutral buoyancy, nor did auriculariae and
bipinnariae. Stability of orientations at neutral buoyancy
was demonstrated by changing the positions of larvae
with a needle and observing their return to their undis-
turbed positions.

The reorientation of some plutei in denser fluids was
caused by a different distribution of excess mass in dense
media. The center of buoyancy was anterior to the center
of gravity when the surrounding fluid was seawater, but

as these plutei sank into denser fluid, the excess density
of the skeleton relative to the fluid decreased, and the
excess density of soft tissues and body cavities ap-
proached zero and then became negative. The center of
buoyancy did not move because the larvae did not
change shape, but the center of gravity (defined in terms
of excess mass) moved as the density of the surrounding
fluid changed. In a denser fluid, therefore, the center of
gravity was displaced anterior to the center of buoyancy,
and the plutei reversed orientation. These plutei were
those with relatively larger body cavities at their posterior
ends or relatively lighter skeletons overall (for examples,
Fig. 1A, C; 4C–H). These plutei did not reverse orienta-
tion simply because they sank into denser fluid than did
those that remained anterior end upward; the plutei that
remained arms upward at neutral buoyancy (*O. acu-
leata, D. excentricus,* and four-armed *S. purpuratus*)
sank into denser fluid than did plutei that reversed their

orientation (*S. franciscanus* and *S. pallidus*) (Table II). Thus the differences among plutei in shape and skeletal development and the depths at which their stable orientation was anterior end upward fit our interpretation of the reorientation.

The auriculariae and bipinnariae did not reverse orientation, but that does not imply greater passive stability than the plutei that reversed orientation because the auriculariae and bipinnariae did not sink as far in the density gradient as did these plutei. Nevertheless, the decalcified four-armed plutei of *T. gratilla* reversed their orientation to anterior end downward at neutral buoyancy, whereas the bipinnariae and auriculariae remained oriented anterior end upward. This suggests that a pluteus without a skeleton may have less vertical stability than the bipinnaria and auricularia. This could result from the distribution of epidermal cells, which form a thick columnar layer at ciliated bands and are very thin elsewhere; the ciliated band is placed more anteriorly in plutei than in auriculariae and bipinnariae. Perhaps the skeleton is necessary for the pluteus form because of its contribution to vertical stability, in addition to its role in body support.

Effect of drag on orientation

So far we have ignored the effects of drag on orientation. Drag on swimming larvae could be important but drag cannot account for the following observations on orientation. (1) Echinoplutei of *S. pallidus* and *S. franciscanus* species reoriented with their arms downward as they sank into denser fluid. If drag were responsible for an orientation with arms upward, the larvae would not have reoriented with arms pointing downward as they continued to sink. (2) All four types of echinoderm larvae passively reoriented when turned with a glass needle even when they were at their depth of neutral buoyancy and were no longer sinking. Drag associated with sinking was not necessary to produce stable orientations.

Discussion

The existence and forms of calcite skeletons in feeding echinoderm larvae pose difficult questions. Why should gelatinous planktonic animals have internal mineral skeletons? Why do these larvae have calcite skeletal elements that are unsuited for supporting body parts? How did the complex skeletons of plutei evolve? Answers to these questions depend on functional consequences of larval skeletons. We have examined the importance of the skeleton in passive orientation with two approaches: (1) experimental removal of skeletons from echinoplutei and (2) comparisons among larval forms. We have examined the role of larval skeletons in passive vertical orientation both directly and by the contributions of skeletons to relative density and sinking rates. In this discussion we first review other functional roles that the larval skeleton may have and then explore ways in which a requirement for passive vertical orientation could influence the form and distribution of the calcite skeleton.

Skeletal support for arms. The primary body cavity of echinoderm larvae contains elastic gelatinous material that appears adequate to oppose muscles and to support convoluted body surfaces (Strathmann, 1989). Is a skeleton really needed for the support of larval arms? Experiments indicate that the skeleton does indeed play a role in the development of arms of echinoplutei, but comparisons show that not all arms require a skeleton and that not all calcite skeletal elements support arms. Experiments by Hörstadius (1939), Okazaki (1956), and others showed that an interaction between skeletal rods and the epidermis is necessary for normal development of arms of echinoplutei, though short and rudimentary arms form without skeletal rods. Our removal of skeletons from plutei confirmed these observations. When living plutei with well-developed arms were decalcified, the arms initially became shorter and thicker than the arms of controls (Fig. 2A–C). Within several days the arms completely disappeared in echinoplutei that did not resecrete a skeleton (Fig. 2D). In echinoplutei that resecreted skeletal rods, the arms formed normally, except that length and direction were usually abnormal (Fig. 2E, F).

The skeleton was necessary to maintain the arms of echinoplutei, but skeletal rods are not necessary to support all arms of echinoderm larvae; asteroid larvae lack a calcite larval skeleton, but many late stage asteroid larvae nevertheless develop arms (Mortensen, 1921, 1938). Also, not all skeletal elements support arms; *e.g.*, parts of the body skeleton of plutei and the posterior ossicles of auriculariae. Thus experiments on echinoplutei indicate that the calcite skeletal rods maintain the larval arms; but in some of the other feeding echinoderm larvae skeletal rods are not necessary for the development or support of arms, and the support of arms is not the function of all skeletal elements.

Skeletal defense against predators. Emlet (1983) determined that the fenestrated skeletal rods of arms were stiffer than necessary to support them against forces generated in swimming. Arms of our decalcified live plutei did not discernibly bend or flex during swimming, though admittedly these decalcified arms were very short. Because many arm rods appeared to be much heavier than necessary for support, Emlet (1983) suggested that the skeleton plays a role in defense against predators. The hypothesis is plausible, but direct evidence is lacking. In laboratory experiments, echinoid prism and pluteus stages, which have skeletons, were less vulnerable to predators than were earlier stages that lacked skeletons (Pennington *et al.*, 1986), but this could

have been from predator avoidance following development of ciliary arrest and reversal at the late prism stage (Rumrill *et al.*, 1985) rather than from skeletal protection, and the skeleton does not prevent ingestion by crab zoeae (Rumrill and Chia, 1986). A defensive role for the larval skeleton is possible but not yet tested.

Vertical orientation. Runnström (1918) and Pennington and Emlet (1986) suggested that the echinopluteus skeleton weights a larva so that it passively orients with its arms upwards. In such an orientation, forward swimming produces upwards movement that counters sinking. We tested this hypothesis by comparing the vertical orientation of control and decalcified larvae. Vertical orientation and stability of both live and dead four-armed echinoplutei was reduced by removal of the skeleton. Although other effects of temporary exposure to low pH or the shape changes could have affected the vertical orientation of decalcified live larvae, such objections do not apply to the orientation of the dead four-armed echinoplutei without skeletons.

Does the larval skeleton contribute a sufficient part of the total excess mass that it could influence passive orientation? For plutei the answer is "yes," because the small skeleton of a pluteus has a large effect on whole body density and sinking rate. At a salinity of 33‰ and temperature of 25°C the density of seawater is 1.02 g/ml. Therefore, for *T. gratilla*, the estimated excess density of the four-armed echinopluteus without a skeleton was roughly the difference between 1.04 and 1.02 g/ml. The additional contribution of the larval skeleton was the difference between 1.06 and 1.04 g/ml. By this estimate, the skeleton of the four-armed echinopluteus contributed half the excess density of the larva. A similar rough estimate based on the difference in densities of prisms and four-armed plutei of *D. excentricus* (Pennington and Emlet, 1986) suggests that the skeleton of some plutei may contribute as much as 77% of the excess density. If the body volume of four-armed *T. gratilla* did not change with the removal of the skeleton (Fig. 1A, B), then the proportion of the total body mass that was skeleton can be estimated as $(1.06-1.04)/1.06$, or only about 2% of the body mass. If the density of the skeleton is the same as the density of mineral calcite (2.71 g/ml), then the volume of the skeleton can be estimated as approximately $[(1.06-1.04)/2.71]$ (body volume), or less than 1% of the volume of the larva. These approximate estimates demonstrate that a skeleton of relatively small mass and volume contributed much of the excess mass of the larval body.

The estimated sinking rates of four-armed plutei of *T. gratilla* suggest an even greater contribution of the skeleton to excess density. For slowly sinking objects of this size and the same shape, the sinking rate should be proportional to the excess density (Vogel, 1981). According to the estimated sinking speeds, the plutei with skeletons sank about 5.5 to 8.4 times as fast as the plutei without skeletons. By this estimate, the skeleton provided up to $7/8$ of the excess mass of the whole larva. The discrepancy between estimates of sinking rates and estimates of density could result from errors in both estimates. The measured sinking rates were quite variable, and the estimate of density of the larval body could have been biased by the intervals between the densities of test solutions. Although the exact contribution of the skeleton to excess mass is uncertain, the estimated contribution of approximately $1/2$ to $7/8$ of excess mass is substantial and indicates that the distribution of skeletal elements is important for the passive orientation of plutei.

Does passive vertical orientation depend on a calcite skeleton for all types of feeding echinoderm larvae? All four types of feeding larvae, even the bipinnariae (with no skeleton), were stable with their anterior ends upward. Also, prior to the deposition of any skeleton, swimming echinoid blastulae and gastrulae swim upward in culture (Lyon, 1906), a presumably passive orientation to gravity. A calcite skeleton can enhance passive vertical orientation, but some degree of vertical orientation and gravitational stability is clearly possible without it.

Does excess density depend on skeletal development for all planktotrophic echinoderm larvae? Or do other features have a greater effect on buoyancy? Comparisons of the varied types of echinoderm larvae showed that larvae with a larger volume of skeleton relative to the volume of soft parts were denser. The plutei were denser than the auriculariae and bipinnariae, and plutei with large skeletal volumes relative to their body volumes were denser than the plutei whose skeletons were relatively small. Also, the passive orientation of plutei with relatively small skeletons was more affected by the distribution of soft parts, as was shown by resting orientation of plutei in solutions denser than their soft parts. Because whole body density increases with the amount of skeleton, there is a functional requirement for the distribution of skeletal parts within the body: if larvae with large skeletons are to maintain passive vertical orientation, the skeleton must have a large posterior component.

Does a skeletal element as small as the posterior ossicle of an auricularia enhance passive orientation? Our observations suggested little contribution of the ossicle of the auricularia of *P. californicus* to excess mass, but we cannot rule out a function in passive orientation. The auriculariae with posterior ossicles had whole body densities similar to those of bipinnariae and of auriculariae without posterior ossicles, and measurements of the ossicles also suggested only a small contribution to excess mass. Most posterior ossicles of *P. californicus* were very flat (Fig. 3D, E, F) and therefore had very small volumes. Nevertheless, the small mass of the ossicle could affect

passive orientation because of its extreme posterior position.

Costs of swimming with a skeleton. Larvae with large excess densities must swim to counter sinking. What energetic cost does this impose? To estimate the work necessary to counter sinking, we have combined our estimates for *T. gratilla* with published data for four-armed *D. excentricus*. If a four-armed pluteus has a volume of 2.6×10^{-6} ml (McEdward, 1984) and an excess density of 0.04 g/ml, then the excess mass is 10^{-7} g, and the downward force ($F = mg$) is 10^{-4} dyne. The product of this force and our estimated sinking velocity of 0.044 cm/s is 4.4×10^{-13} J/s, the rate of work done against gravity. A similar calculation for decalcified plutei with 0.02 g/ml excess density, that sank at 0.0052 cm/s, gives 0.26×10^{-13} J/s, so that the skeleton is responsible for 94% of the work done against gravity. This may be an exaggeration because of the discrepancy between our estimates of sinking rates and excess densities, but even if sinking rates differed by only a factor of 2, as expected from our estimate of excess density, the skeleton would be responsible for 75% of the work done.

However, the total work against gravity appears to be trivially small when compared to total respiratory rate of the larva. An estimate of metabolic capacity for a four-armed pluteus can be converted to a rate of oxygen consumption (McEdward and Strathmann, 1987) and then converted to energy units (4.65×10^{-11} mol O_2/h/larva $\times 22.4 l O_2$/mol $\times 4800$ cal/l O_2; conversion factors from Schmidt-Nielsen, 1979) to get 5.8×10^{-9} J/s. The work done against gravity is thereby estimated to be less than 0.01% of the larva's total energy expenditure. The errors in the estimates used in this calculation are undoubtedly large, and the efficiency of the work of swimming is unknown, but the energetic cost of swimming with a skeleton appears to be small. Calculations of work done by swimming copepods (Vlymen, 1970; Alcaraz and Strickler, 1988) and ciliates (Fenchel, 1987) are also a very small part of energy expenditure, as estimated from oxygen consumption. Swimming to counter an increased sinking rate caused by the skeleton apparently does not present an energetic problem for the larvae.

Another consideration is the effect of excess density on rate of ascent. Estimated swimming speeds for feeding larval stages of echinoderms are commonly about 0.3 to 0.5 mm/s (Strathmann, 1971). Konstantinova (1966) estimated 0.3 mm/s for a bipinnaria, but Konstantinova's estimate of 0.1 mm/s for an ophiopluteus appears to be an underestimate. In extensive but unpublished observations, H.-t. Lee measured horizontal swimming speeds of 0.3 to 0.5 mm/s for bipinnariae, up to 0.4 mm/s for auriculariae of *P. californicus*, 0.4 mm/s for two-armed ophioplutei of *O. aculeata*, and 0.4 to 1.9 mm/s for four- to eight-armed echinoplutei of *D. excentricus* and *S.*

droebachiensis. The sinking rates of 0.3 and 0.4 mm/s measured for dead four-armed plutei of *T. gratilla* are comparable to these swimming speeds. Plutei would need more propulsion than bipinnariae or auriculariae to achieve the same rate of vertical ascent.

Ultimately, costs of maintaining depth might be independent of excess mass. Echinoderm larvae swim when they feed because the ciliated band produces currents for both swimming and feeding (Strathmann, 1971). If larvae must feed much of the time and if sinking speeds are less than or equal to swimming speeds produced during feeding, then the larvae need only to orient upward to maintain or decrease their depth.

Passive orientation and skeletal form. Because some types of feeding echinoderm larvae orient to gravity without any skeleton, it seems doubtful that the elaborate skeletons of plutei evolved primarily to produce passive vertical orientation. Nevertheless, if vertical orientation is important, its requirements constrain the form of the pluteus skeleton and may explain many features of pluteus morphology. A larger and more elaborate posterior body skeleton is correlated with a larger anterior arm skeleton, as shown by examples in Mortensen (1921, 1938). The thickened posterior body rods in early echinoplutei of the Strongylocentrotidae and Echinidae and the posterior rods of spatangoid plutei may serve as a counterweight for the larval arms.

Similarly, juvenile skeletal elements may be constrained to develop in positions that maintain passive orientation. The asteroid, echinoid, and ophiuroid juvenile rudiments develop in a posterior position.

A role of the skeleton in passive orientation also suggests a functionally advantageous and simple first step in the evolution of larval skeletons. All that is required for passive orientation is a posterior position of the skeletal element; the posterior ossicles in auriculariae may be an example. In contrast, a supporting skeleton must develop as a system of rods favorably placed for supporting muscles or extensions of the ciliated band. Improvements in the skeletal form for support of muscles or projecting arms may have evolved after early formation of calcite ossicles was established.

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